Evaluation of nootropic activity of combination of *Tinospora cordiofolio Willd. Miers* and *Centella asitica Linn.* and its formulation development.

Synopsis

Submitted in

Partial fulfillment for degree of

Doctor of Philosophy

(Pharmaceutical Sciences)

(Quality Assurance)

Supervised and Guided by

JV'n Prof. (Dr.) Dharmendra Ahuja

Submitted by

JV'n Abhishek Sharma

JVR2173

Department of Pharmaceutical Sciences

JAYOTI VIDYAPEETH WOMEN'S UNIVERSITY (JVWU)

Jaipur, Rajasthan (INDIA)

2021-2022

CONTENTS

S.NO.	CONTENTS	PAGE NO.
1.	INTRODUCTION	3
2.	HYPOTHESIS	7
3.	REVIEW OF LITERATURE	8
4.	AIM AND OBJECTIVE	13
5.	METHODOLOGY	14
6.	REFERNCE	16

➢ INTRODUCTION

Definition & concept of “Nootropic” / memory enhancing drug was first introduced in 1972 by C.E. Guirgea, the lead and principle Piracetam researcher of UCB Pharmaceutical Company (Belgium) who launched Piracetam. “Nootropic" term was coined by him for the first time from italic words "noos" which means mind & "tropein" which means to turn towards i.e. enhancement of learning and memory.[1] Compounds having ability to boost cognitive abilities and enhance mental performance is known as Nootropics (Common terminologies of Nootropic are smart drug, memory enhancing drug, brain booster), although some people with history of mental disorder may be susceptible to its adverse effects. Nootropics are compounds that increase attention, concentration, memory, and motivation overall mental abilities.[2,3] The first nootropic agent which was discovered was Piracetam.

Main features that highlight a nootropic drug are as:-

- Enhancement of learning, memory and concentration.
- Protection of brain against various injuries (chemical or physical).

Pharmacological properties of Nootropics:

Various studies have been conducted for modes of action of the nootropics, which has revealed various pharmacological effects. Although there might be no single dominant mode of action which is shared by the whole drug class. All these drugs however influence cholinergic functions. By enhancing high affinity choline uptake these drugs facilitate the production of acetylcholine. There is a serious decline in acetylcholine receptors in normal aging in humans. Nootropics elevates the density of frontal cortex acetylcholine receptors by 30- 40% restoring the levels of acetylcholine in brain.[1]

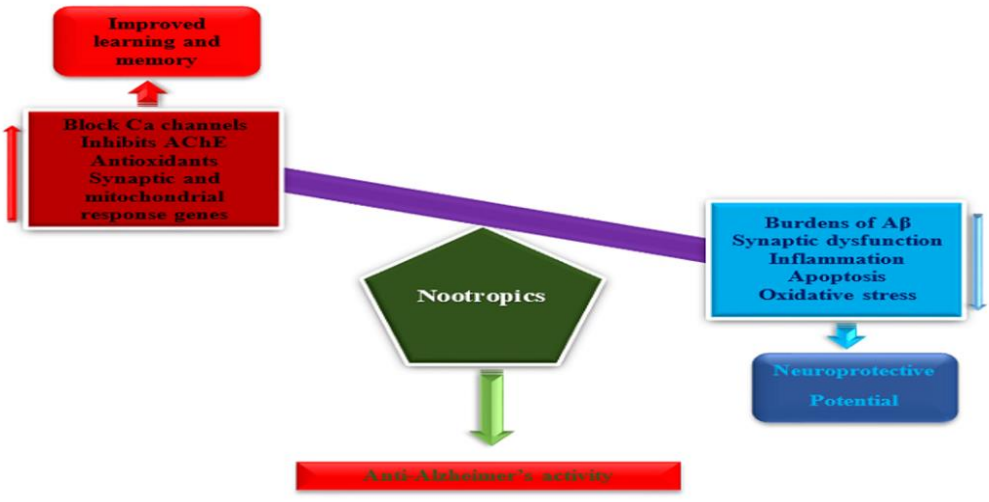


Figure-1

Figure-1: Schematic representation of mechanism of action of nootropics: Nootropics improve learning and memory by blockage of Ca channels, inhibition of AChE activities thus increases the level of antioxidants and increase synaptic & mitochondrial response of the genes. Nootropics are the agent that provides neuroprotective potentials by reducing the burdens of Aβ accumulation, synaptic dysfunctions, oxidative stress, inflammation & apoptosis. Nootropics can be classified into two categories: Naturally occurring nootropic, such as *Centella asiatica*, *Tinospora cordifolia, Ginkgo biloba* among others and synthetic nootropic, laboratory created compounds such as Piracetam, racetams and modafinil.[2,3]

Recently, a tremendous urge to explore medicinal plants globally for improving cognitive function has gain growth owing to their less adverse effects. In Ayurveda there are number of herbs that are known for their nootropic activity and promising effects as well as their multi-dimensional utility in various conditions. [3]

Among multifold benefits Mandukaparni (*Centella asiatica Linn*.), *Guduchi* (*Tinospora cordifolia* (Willd.) Miers) are mentioned with wide range of applications on different systems. Choice of Plants are as Mandukaparni (*Centella asiatica Linn*.), *Guduchi* (*Tinospora cordifolia).*

Mandukaparni (Scientific name: *Centella asiatica Linn*.) is a prostrate, stoloniferous perennial herb rooting at nodes [Figure 2]. From ancient time in the ayurveda, fresh juice of whole plant is used for therapeutic purposes as Medhya (cognitive enhancer). The major constituents are saponin, medacoside, asiaticoside, medacassoside, asiatic acid, new triterpenic acid. They act on behaviour and known to being neuroprotectives brain growth promoter. Memory impairment induced by scopolamine inhibited by *Centella asiatica* through the inhibition of AChE.[3] In India herbal formulation as a tonic which is used to increase memory power, immunity booster, anti-stress substance, anti-epilepsy and anti-anxiety. *Centella asiatica* acts as an anti-oxidant against lead acetate induced neurotoxicity, amnesia and ideas recalling. In early postnatal developmental stages, *Centella asiatica* can produce long-lasting beneficial effects by mediating effect on the cholinergic system and as well as on neuronal morphology and thus improving the endogenous antioxidant enzymes in the rat brain. [4]

Figure-2 *Centella asiatica*

Guduchi (*Tinospora cordifolia):* Guduchi (*Tinospora cordifolia* (Willd.) Miers) is a large glabrous, deciduous, climbing shrub which is found throughout the tropical India from the family Menispermaceae [Figure 3]. Juice of whole plant is used therapeutically as Medhya (cognitive enhancer). Guduchi is also used in various form of decoction, powder and Satwa (starch extract of stem). Root of this plant is also known for its anti stress, anti-malarial and anti-leprotic activities. Chemical constituents classes of *Guduchi* are alkaloids, glycosides, steroids, diterpenoid lactones, sesquiterpenoid, phenolics, aliphatic compounds and polysaccharides. Neuroprotective and ameliorative properties is due to their antioxidant and trace element contents of *Tinospora cordifolia*. *Tinospora cordifolia* is rich source of trace elements such as Copper & Zinc which act as great antioxidants source and protects the cells from damaging effects of oxygen radicals generated during activation of immune. It also increases the blood profile and has lead scavenging activity. *Tinospora cordifolia* has been proved for its multifold action, it possess learning and memory enhancing, antioxidant, and anti-stress activity. *Tinospora cordifolia* enhances the cognition in normal and cognition deficits animals in behavioural test Hebb William maze and the passive avoidance task. Mechanism of cognitive enhancement is due to immunostimulation and increase in synthesis of acetylcholine, thus enhances the cognition. Multifold actions of Guduchi may be attributed to its antioxidant and immunomodulatory properties.[3]

In Indian *Tinospora cordifolia* plays an important role and termed as an important drug which is used in medicine as antioxidant, antistress properties for treating depression, Alzheimer disease, improving cerebral ischaemia, rejuvenator or adaptogen, immunomodulatory, anti-inflammatory, anti-hyperglycaemic, antispasmodic, antiulcer and attention-deficit hyperactivity disorder, oxidative stress injury and regulation of

cytokines, memory and concentration enhancement by inhibiting reuptake of amines in brain and increased levels of norepinephrine (NE), serotonin (5-hydroxytryptamine or 5-HT), dopamine (DA), and decreased levels of gamma-aminobutyric acid (GABA) have been demonstrated. In herbal formualtion, *Tinospora cordifolia* works as synergistically for making the important component of polyherbal formulations for treating depression and several disease. [4]

Figure 3 *Tinospora cordifolia*

➢ HYPOTHESIS

Plants are the vital source of primary metabolites such as amino acids, fats, sugar along with secondary metabolites like alkaloids, Saponins, flavonoids, glycosides etc. which shows pharmacological activity/actions which depends on the drug delivery system. A polyherbal formulation shall be developed after successful evaluation of the pharmacological activity of the polyherbal combination.

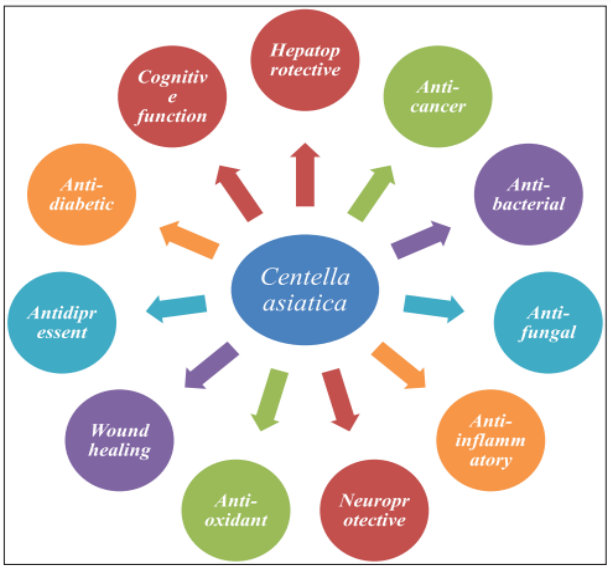
Polyherbal formulation of *Centella asiatica* and *Tinospora cordifolia* for memory enhancing property, the evaluation of the nootropic activity shall be checked on the animal, thereafter formulation development shall begin. These both herbs are termed as *Medhya Rasayana* in Ayurveda which means *Medhya Rasayanas* are group of medicinal plants which is described in Ayurveda (Indian system of medicine) with multi-fold benefits and no adverse effects.

On the basis of this hypothesis, using these polyherbal combinations, nootropic activity / memory enhancing activity shall be demonstrated thereafter formulation development shall be initiated.

➢ REVIEW OF LITERTURE

✓ Nootrpic Activity:

- In Ayurveda, "medhyarasayanas" terminology is defined which represents herbal therapeutics that can boost memory, restore cognitive deficits and improve mental function thus improving the concentration. "Brahmi" is a traditional herb that corresponds to two plants, *Bacopa monnieri* and *Centella asiatica*, research clearly indicate that both plants have nootropic activity which possess neuroprotective properties, with therapeutic implications for patients with memory loss.[5]
- Evaluating nootropic effect of *Centella asiatica* in three months old male Swiss albino mice. An aqueous extract of *Centella asiatica* were injected orally with graded doses (200, 500, 700, 1000 mg/kg body weight) for 15 days to select an effective dose for nootropic studies. Evaluation of nootropic effect was tested in radial arm maze to assess the learning and memory performance. Based on the observation, mice treated orally with 200 mg/kg of *Centella asiatica* for 15 days from day 15 to day 30 post partum (p.p.) and the nootropic effect evaluated on the 31st day and 6 months p.p. The behavioral study (open field, dark/bright arena, hole board and radial arm maze tests), biochemical study (acetylcholine esterase activity) and histological studies (dendritic arborization) were carried out. Performance of juvenile and young adult mice was improved significantly in radial arm maze and hole board tests, but locomotor activity did not show any change compared to control. Increased acetylcholine esterase activities in the hippocampus were observed. Dendritic arborization of hippocampal CA3 neurons were increased in terms of intersections and branching points, both at 1 month and 6 months. Results show that treatment with *Centella asiatica* extract can influence the neuronal morphology and promote the higher brain function of juvenile and young adult mice during postnatal developmental stage.[6]
- *E. alsinoides* & *C. asiatica* are widely used in traditional Indian medicine as the neuroprotective agent and also found promising effects over immunomodulatory activity, inflammatory diseases and wound relieve/healing. Neuroprotective effect of both plants extracts found due to inhibition of AChE (Acetylcholinesterase) activity and improve the spatial memory formation.[7]



Various Pharmacological activities of *Centella asiatica*[8]

- Evaluation of the nootropic property of n-butanolic (TBF) fraction of ethanolic extract-stem of *Tinospora cordifolia* which contains saponin. The fraction was administered at 100 mg/kg and 200 mg/kg and nootropic effect were evaluated using mice in the elevated plus maze, the evaluation is done to determine the transfer latency on day-2 and Day-9. Similarly passive avoidance test were carried out in mice to evaluate the step down latency on day-2 and Day-9. Recognition test carried out on the object to determine the recognition index. Scopolamine (1 mg/kg) was used as an amnesic agent whereas piracetam (250 mg/kg) was used as a standard nootropic drug. Results showed significant increase in transfer latency by TBF on day -2 and Day-9. TBF also significantly increases step down latency, object recognition index. Scopolamine was used to produce amnesia which significantly decreases the transfer latency, step down latency, recognition index of the object. The TBF antagonised the amnesic effects of scopolamine in the experimental models. Estimation of acetylcholinesteras (AchE) concentration in mice brain was evaluated after 7-days treatment with TBF which produces the Anti-cholinesterase activity. Result shows decrease in AchE concentration which indicates involvement of cholinergic system in nootropic activity of TBF. [9]
- *Tinospora cordifolia*, an ancient Indian medicinal plant, reported to have several beneficial effects on disorders such as rheumatism, peptic ulcer, hepatobiliary disorders and several infectious diseases etc. The plant enhances cognition in normal rats and successfully overcomes cyclosporine induced memory deficit. Methods: 30 healthy volunteers of age group around 18-30 years were received

pure aqueous extract of Tinospora cordifolia (500 mg) and placebo (no active) for 21 days in a double blind, randomized and in controlled placebo design. Memory & Learning enhancement was assessed by subjecting the volunteers to a series of psychological tests that aimed at studying logical memory, visual memory, verbal memory, attention and concentration.

Results. *Tinospora cordifolia* shows significant ($p < 0.05$) increase in the test scores of 'verbal learning and memory' (control -1.2±1.9, drug 6.9±2.5) and logical memory (control 5.1±6.1, drug 26.6±6.7). No significant adverse effects were reported during *Tinospora cordifolia* treatment. Conclusion: based on the observation it is concluded that *Tinospora cordifolia*, 500 mg daily dose, enhances verbal learning memory and logical memory (of immediate and short term type) compared to placebo in healthy volunteers. [10]

- In Ayurvedic texts Medhya drugs are mentioned for their multifold benefits, enhancement of cognition which acts on psychological co-morbidities like stress, depression and agitation. Guduchi (*Tinospora cordifolia*) is known as one of the medhya Rasayana which possess multi-dimensional actions and also have an influence on mental health. [11]

✓ **Hypoglycaemic Activity:**

- In Indian Ayurvedic medicine *Tinospora cordifolia* is widely used for treatment of diabetes mellitus. Upon oral administration of aqueous extract of *T. cordifolia* root (TCREt) to alloxan diabetic rats causes a significant reduction in blood glucose and brain lipids level. The aqueous extract causes increase in body weight, total haemoglobin and hepatic hexokinase. It was observed that root extract decreases hepatic glucose-6-phosphatase and serum acid phosphatase, alkaline phosphatase, and lactate dehydrogenase in diabetic rats. Hence aqueous extract of *T. cordifolia* root shows hypoglycaemic and hypolipidaemic effect. [12]
- Administration of aqueous extract of *C. asiatica* leaf to diabetic rats maintain normal ATPases activity levels and thus prevent the increasing levels of inflammatory and oxidative stress markers in the hippocampus. Diabetic rats treated with aqueous extract *C. asiatica* leaf shows lesser signs of histopathological changes in the hippocampus. *Conclusions*. Aqueous extract *C. asiatica* leaf protects the hippocampus against diabetes-induced dysfunction which could help to preserve memory in such condition. [13]

- ✓ **Antibacterial Activity:**
  - Antibacterial activities were observed using crude extracts of *Centella asiatica* (Linn.) Urban extracted with ethanol or water were tested against 30 isolates of S. aureus from milk samples of dairy cows were tested by disc diffusion test and observed that both ethanol extracts as well as water extracts had average inhibition zones ranged from 6.44-6.49 and 6.54-17.72mm diameter, respectively. To determine the minimum inhibitory concentration (MIC), modified resazurin microtiter-plate was used and the minimum bactericidal concentration (MBC) was determined by touching the loop from each well of MIC plate and streaking it on a mannitol salt agar which showed that the ethanol extracts had an MIC50 value of 8 mg/mL, the water extracts of leaf powder had an MIC50 value of 32 mg/mL, and similarly water extracts of fresh leaves had an MIC value of 32- 256 mg/mL. The ethanol extracts had an MBC value of 16 mg/mL whereas water extracts could not produce the results for kill S. aureus. Based on the observation it is concluded that ethanol extracts had more potential antibacterial activity than the water extracts. [14]
  - Antibacterial activity were observed from the ethanol, aqueous and chloroform extracts from the stems of *Tinospora cordifolia* was studied using disc diffusion method, the study against E.coil, Salmonella typhi (Gram-negative), Proteus vulgaris, Enterobacter faecalis, Staphylococcus aureus and Serratia marcesenses (Gram-positive). Results suggested that ethanolic extract from the stems of *Tinospora cordifolia* has significant antibacterial activity against tested bacteria. [15]
- ✓ **Anti-fungal activity:**
  - *Anti-fungal activity of* crude methanol extract of *Centella asiatica* was found to be the most effective against fungal activity. Broth dilution and disc diffusion was prepared and compared to disc diffusion, broth dilution was observed as more appropriate method for quantitatively determination of the anti-Candida activity of plant extract, whereby the MIC values of crude extracts was determined. The result from disc diffusion assay observe that assay demonstrates that plant extracts have an inhibitory effect. However, the results observed from the broth dilution method reveals that *Centella asiatica* crude methanol extract has lower MIC values, which means that *Centella asiatica* has an anti-fungal effect. [16]

✓ **Analgesic, Anti-Inflammatory and Anti-Pyretic Activities:**

- Significant Analgesic effects were observed from the extract of *T. cordifolia* in a dose-dependent manner which is tested in the three pain models. The extract also exhibited significant anti-inflammatory and antipyretic effects. An anti-inflammatory effects in the carrageenan-induced inflammation test and antipyretic effects in the brewer's yeast-induced pyrexia test in dose--dependent manner compared to the effects observed in the control group animals. Based on the study it can be concluded that *T. cordifolia* extract possess analgesic, anti-inflammatory and anti-pyretic effects. [17]
- Anti-inflammatory activity is observed in the extract of *Centella asiatica.* For anti-inflammatory activity the inhibition of hypotonicity induced HRBC membrane lysis was taken. Membrane stabilization for methanolic extracts and Diclofenac sodium were performed at several concentrations. The maximum membrane stabilization from extract of *Centella asiatica* were observed to be 94.97 % at a dose of 2000µg/ml. Hence based on the observation test support the isolation and the use of active constituents from *Centella asiatica* in treating inflammations. [18]

➢ AIM AND OBJECTIVE

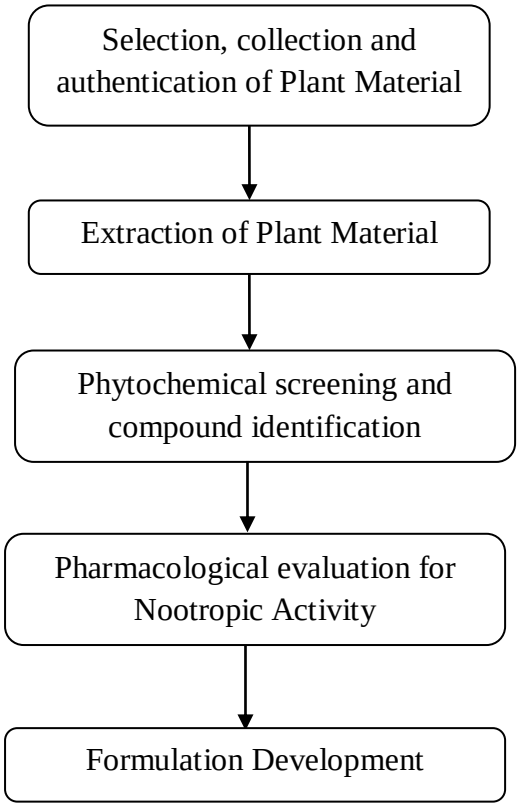
In modern medical science, the symptoms are frequently appears in various disease or disorders as a result of different types of deficiencies now a day's related to modern medical therapies includes toxicity, ADR, etc. and several health issues will generate toxic effects.

Due to these disadvantages of modern allopathic therapy, the way to increase towards herbal drugs.

The current research work includes identification, extraction, isolation, characterization, pharmacological evaluation and formulation development of memory enhancing activity (Nootropic activity) of *Centella asiatica* and *Tinospora cordifolia*.

- To study Plant material for identification and authentication.
- To study phytochemical screening and characterization of compounds.
- To evaluate the pharmacological action of the active ingredient on the test animals.
- To develop suitable polyherbal formulation.

➢ METHODOLOGY


Selection, collection and authentication of Plant Material
Extraction of Plant Material
Phytochemical screening and compound identification
Pharmacological evaluation for Nootropic Activity
Formulation Development

Methodology: Selection and collection of the Tinospora cordifoli and Centella asiatica may be done form Plant nursery / Ayurvedic farm / any other source. Upon collection of the plants, authentication of plant material may be done from the head of department of the botany / botanist. After successful authentication of the plant material, extraction (may be aqueous extract / alcoholic extract) of the plant of interest shall be performed as per the standard method / any other suitable method. Extract obtained hereby are explored to Phytochemical screening and compound identification by suitable method. After screening and identification, pharmacological evaluation of the extract obtained from both the plants is required to be checked. Evaluation may be performed using mice / any other suitable animal. Suitable experiment which may include elevated plus maze, radial arm maze & morris water maze etc may be designed for the evaluation of nootropic activity on suitable animal. Demonstrate the experiment to conclude the result and findings in combination of the plant. Conclude the finding as per the methodology used. Finding shall conclude the pharmacological activity of the plants in combination.

Finally after successful evaluation of the pharmacological activity, suitable formulation (may be liquid, granules etc) shall be developed in combination.

Importance / Society application

The utilization of herbs as a drug has long and ancient history throughout in the world to cure, treat, diagnose various diseases and herbal formulations also are found in pharmacopoeia. With less or no toxic effect herbal formulation are gaining interests in the world. Herbal formulations or polyherbal formulations have been the most effective treatment for several disease conditions. Many studies have been reported to prove the efficiency of herb-herb combinations. In comparison with single drugs, drug combinations have shown a more promising effect in the treatment of disease. This drug combinations conception has been well established in many countries and extraordinary success has been reached. It is an attempt to develop the use of polyherbal formulation for the memory enhancing activity i.e. Nootropics activity.

For example we can say that in recent years especially in COVID'19 Pandemic there has been more interest developed/generated for herbal formulations worldwide which is specially due to multi-fold benefits of herbal formulation or polyherbal formulation.

With the use of herbal formulation, any kind of disease/disorders can be cure therapeutically without showing toxic effects.

➢ REFERENCE

1. Balaraman, R., & Shingala, J. (2002). Molecule of the millennium. Indian journal of pharmacology, 34(6), 439-440.
2. Chiroma, S. M., Taib, C. N. M., Moklas, M. A. M., Baharuldin, M. T. H., Amom, Z., & Jagadeesan, S. (2019). The use of nootropics in Alzheimer's disease: is there light at the end of the tunnel? Biomedical Research and Therapy, 6(1), 2937-2944.
3. Kulkarni, R., Girish, K. J., & Kumar, A. (2012). Nootropic herbs (Medhya Rasayana) in Ayurveda: an update. Pharmacognosy reviews, 6(12), 147.
4. Goel, B., & Kumar Maurya, N. (2019). Memory booster herb (natural cognitive enhancers): An overview. International Journal of Physiology, Nutrition and Physical Education, 4(1), 975-9.
5. Shinomol, G. K., & Bharath, M. M. (2011). Exploring the role of "Brahmi" (Bacopa monnieri and Centella asiatica) in brain function and therapy. Recent patents on endocrine, metabolic & immune drug discovery, 5(1), 33-49.
6. Rao, S. B., Chetana, M., & Devi, P. U. (2005). Centella asiatica treatment during postnatal period enhances learning and memory in mice. Physiology & behavior, 86(4), 449-457.
7. Yadav, M. K., Singh, S. K., Singh, M., Mishra, S. S., Singh, A. K., Tripathi, J. S., & Tripathi, Y. B. (2019). Neuroprotective Activity of Evolvulus alsinoides & Centella asiatica Ethanolic Extracts in Scopolamine-Induced Amnesia in Swiss Albino Mice. Open access Macedonian journal of medical sciences, 7(7), 1059.
8. Prakash, V., Jaiswal, N. I. S. H. I. T. A., & Srivastava, M. R. I. N. A. L. (2017). A review on medicinal properties of Centella asiatica. Asian J Pharm Clin Res, 10(10), 69.
9. Une, H. D., Ejaj, M. A., & Tarde, V. A. (2014). Nootropic activity of saponins obtained from Tinospora cordifolia stem in scopolamine induced amnesia. Int J Pharma Res Rev, 3(2), 28-35.
10. RAO, Y., KUMAR, B., & BAIRY, L. (2004). Efficacy of Tinospora cordifolia on learning and memory in healthy volunteers: a double-blind, randomized, placebo controlled study.
11. Pansare, T. A., Pranita, D., & Archana, B. (2018). Review on Medhya (Intellect Promoting) action of Guduchi [Tinospora cordifolia (Wild) Miers].

12. Stanely, P., Prince, M., & Menon, V. P. (2000). Hypoglycaemic and other related actions of Tinospora cordifolia roots in alloxan-induced diabetic rats. Journal of ethnopharmacology, 70(1), 9-15.
13. Giribabu, N., Srinivasarao, N., Swapna Rekha, S., Muniandy, S., & Salleh, N. (2014). Centella asiatica attenuates diabetes induced hippocampal changes in experimental diabetic rats. Evidence-Based Complementary and Alternative Medicine, 2014.
14. Taemchuay, D., Rukkwamsuk, T., Sakpuaram, T., & Ruangwises, N. (2009). Antibacterial activity of crude extracts of Centella asiatica against Staphylococcus aureus in bovine mastitis. Kasetsart Veterinarians, 19(3), 119-128.
15. Jeyachandran, R., Xavier, T. F., & Anand, S. P. (2003). Antibacterial activity of stem extracts of Tinospora cordifolia (Willd) Hook. f & Thomson. Ancient science of life, 23(1), 40.
16. Bhuyar, P., Maniam, G. P., & Govindan, N. (2021). Isolation and characterization of bioactive compounds in medicinal plant Centella asiatica and study the effects on fungal activities. Journal of microbiology, biotechnology and food sciences, 10(4), 631-635.
17. Hussain, L., Akash, M. S., Ain, N. U., Rehman, K., & Ibrahim, M. (2015). The analgesic, anti-inflammatory and anti-pyretic activities of Tinospora cordifolia. Advances in Clinical and Experimental Medicine, 24(6), 957-964.
18. Chippada, S. C., Volluri, S. S., Bammidi, S. R., & Vangalapati, M. (2011). In vitro anti-inflammatory activity of methanolic extract of Centella asiatica by HRBC membrane stabilisation. Rasayan J Chem, 4(2), 457-60.